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March 25, 1996

Dear Professor Lederberg:

Thank you for your letter of March, 1996. I sent you an e-mail message this morning. I enclose a set of reprints and a copy of the e-mail message:

The project on fungal viruses has been my "pet" project for sometime now. We have recently made considerable progress in the molecular characterization of the viruses and we are applying molecular approaches to address the interesting biological features of this fungal-virus system

I'll respond to the points you raised in your letter in the following:

Transmission experiments using virus-free fungal protoplasts and purified virions proved to be very inefficient, as I discuss in the enclosed reprints of our work on transmission of the mycoviruses infecting the plant pathogenic fungus "*Helminthosporium victoriae*". The transmission work was published in book chapters that are based on "Symposia Proceedings". I didn't publish these studies as Journal articles because I wanted first to optimize the conditions of the infectivity assays. This proved to be time-consuming.

We have recently completed the molecular characterization of the viruses involved and the virus system is very similar to the yeast killer system (A helper virus that belongs to the family Totiviridae and associated satellite dsRNAs). We have constructed full length cDNA clones of the viral and satellite dsRNAs associated with diseased isolates of the fungus. With the availability in our laboratory of full length cDNA clones and of efficient DNA-mediated transformation system for *H. victoriae*, we have resorted to molecular biology techniques in order to study the etiology of the disease phenotype. Using such a molecular approach, it has been

unequivocally established that viral dsRNA is the causal agent of hypovirulence in the chestnut blight fungus

My thoughts pertaining mechanisms involved in "segregation" during protoplast formation and generation are as follows: protoplasts originating from apical cells in young hyphae may be virus-free or in case of double infections may contain only one of the viruses. The practice of hyphal-tip subculture has been used in curing virus-infected fungal cultures.

Examples of "cure" as a result of chemical treatment:
Cycloheximide has the most commonly used chemical for curing virus-infected fungal cultures. Cycloheximide is known to inhibit virus dsRNA synthesis selectively in fungal hosts.

References:
Fink, G. R. and Styles, C. A. 1972. PNAS 69, 2846-2849
Sweeney et al. 1976. Genetics 84, 27-42.
Lemke et al. 1973. J. Gen. Microbiol. 76, 285
Fulbright, D. W. 1984. Phytopathology 74, 722-724.

We are preparing a paper on the molecular characterization of the 190S virus of *Helminthosporium victoriae* and I will be glad to send you a pre-print if you so desire.

I have recently wrote a rather lengthy review article on recent developments in fungal virology (unfortunately I am out of reprints):

Ghabrial, S. A. 1994. New developments in fungal virology. Adv. Virus Res. 43:303-388.

With best regards.

Sincerely yours,

S A Ghabrial

Said A. Ghabrial